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TITLE: Semi-Synthesis and In-vitro Anticancer Evaluation of Derivatives of a New Microtubule Poison with a Taxol-Like Mechanism

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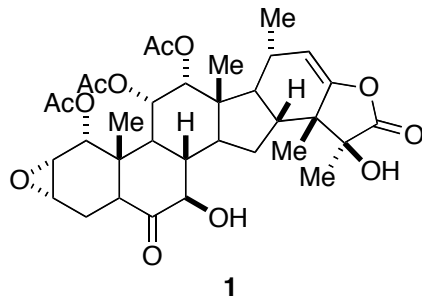
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14. ABSTRACT: Taccalonolides are plant-derived steroids which act as microtubule poisons on mammalian cells with a taxol-like mechanism. This makes them potentially useful lead compounds for the development of anti-cancer agents, specifically breast cancer. Natural taccalonolide A was modified through selective chemical reactions at various sites in the molecule in preparation for biological tests that will compare the biological effects and potency of the derivatives with that of the starting material. These studies will allow the identification of those parts of the structure of taccalonolide A that are important for its biological activity. Work towards this goal is subject of this report.					
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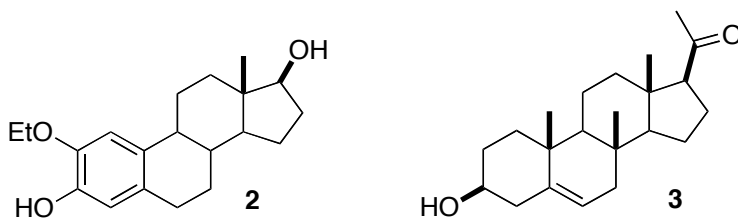
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Introduction

Under prior funding in collaboration with a molecular pharmacologist at the Cancer Research Center of Hawaii we had discovered an unusual plant steroid, taccalonolide A, **1**, from the Asian medicinal plant *Tacca chantrieri*. This compound has microtubule stabilizing properties in cell cultures of mammalian cells similar to that observed for taxol. This effect can be visualized in an indirect immunofluorescence assay that our collaborator had developed.



At the time we discovered the taxol-like activity of taccalonolide, this was the first report of a steroid-like material to possess antimicrotubule properties. However, in the time between our discovery and publication describing the taccalonolide molecular pharmacology [1], another report appeared describing a taxol-like activity for synthetic steroids related to estradiol [2], e.g. compound **2**, and another one describing stimulation of microtubule assembly by pregnenolone **3** [3]. This was followed shortly thereafter by a report on virtual screening of a steroid structure library for potential tubulin binders and the identification of such a derivative [4].

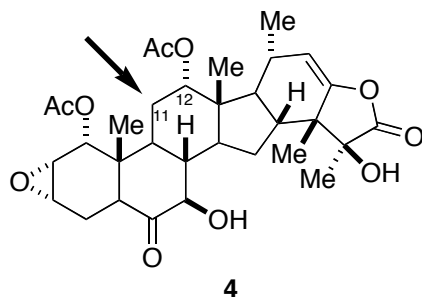


Given the significant structural differences between taccalonolide A and the synthetic compounds described in the latter reports, we set out to pursue a program of structural modification of taccalonolide A in order to identify some of the pharmacophoric elements of the plant natural product. This work was to be based on the semisynthetic modification of taccalonolide A isolated from plant material. Additional derivatives we hoped to obtain through extraction of plant material of other *Tacca* species.

BODY

Our structure modification work was to be guided by biological assays using the immunofluorescence assay with the aid of our colleagues at the Cancer Center. Unfortunately, within five months of starting our work under the present grant our collaborator announced her resignation from the University of Hawaii. Although we had hoped that another molecular pharmacologist would succeed her who would be willing to collaborate with us on this project, this did not come to pass.

We had first reported that the plant did not contain other compounds related to taccalonolide A. This as it turns out was erroneous and the difference to the results reported in ref. 1 was traced to a crystallization step, which apparently efficiently removed a contaminant later identified as taccalonolide E **4**, the C-11 desacetoxy derivative of **1**. We had monitored the crystallization with normal phase chromatography by which **1** and **4** are separated only with great difficulty and hence had no indication that two compounds were present. Our former collaborators isolated **4** by reverse phase HPLC by which the two compounds can easily be separated. We were alerted to this only once the publication describing the Taxol-like activity of the taccalonolides appeared in print in 2003 when we were well into our structure modification program.



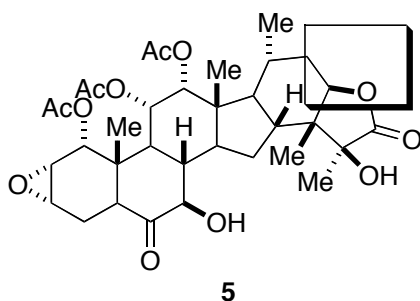
This monodeoxygenated derivative of **1** turns out to be more potent than taccalonolide A is. This in turn suggests that modification of the C-11/C-12 position should be a prime target for our efforts. In our proposal we had envisioned that modifications of the substituents in ring C might be achievable by way of enzyme-catalyzed reactions. Enzymes appeared to be the only reagents that might possess the selectivity to differentiate between these two acetates and the C-1 acetate as well as the sensitive enolester in ring F. We had proposed to employ the cross-linked enzyme crystals commercially available from ALTUS Biologics at the time. However, by the time we were ready to employ this technology, the company had changed its business model and was no longer selling the enzymes. It had turned itself into a contract research company developing enzyme catalysis solutions for corporate clients and has turned, as of late, into a biopharmaceutical company.

Our attempts to effect the selective hydrolysis of the C-11/C-12 acetates in ring C using enzyme catalysis in aqueous buffer solution failed completely as indicated by low substrate recovery and an inability to monitor the reaction progress properly. In conversations with industrial scientists it was suggested that we use deproteinization

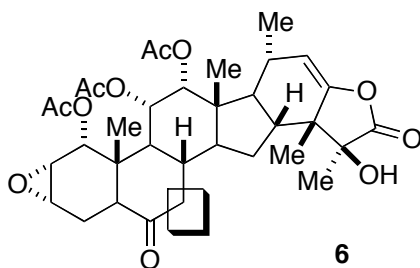
procedures commonly used in *in vitro* drug metabolism studies combined with LCMS to analyze such reactions. At the time the UH chemistry department did not have an LCMS instrument and this was not an option. This deficiency prompted us to pursue the acquisition of such an instrument and as of Fall 2005 we have one running now, albeit too late to benefit this project.

In **Task 2** and **Task 3** of our proposal we had proposed to prepare a total of nine derivatives through modification of single functional groups present in **1**. Most of the reaction conditions were developed on simpler model compounds available in a few steps from commercially materials.

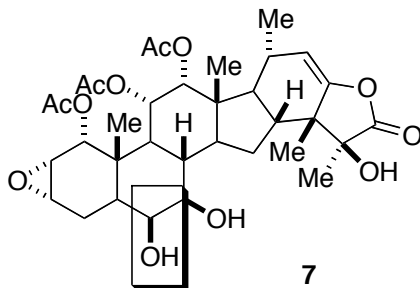
Thus catalytic reduction reduced off the enol ester function in ring E of **1** to yield compound **5** of the indicated stereochemistry.



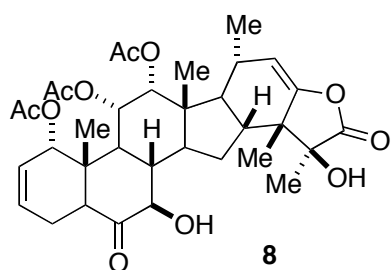
Samarium diiodide was used to deoxygenate C-7 after activation of the alcohol as the triethylsilyl ester to yield **6** after deprotection. Other silyl groups also worked, but triethylsilyl was the best compromise in terms of yield, reaction rate and stability.



Sodium borohydride reduction of the ketone yielded the *cis* diol **7** with an axial alcohol at C-6.



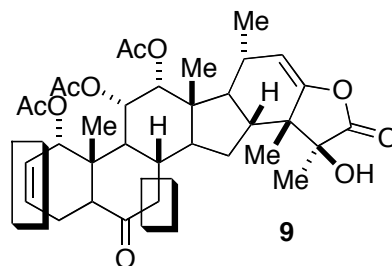
The removal of the epoxide proved more challenging than we had assumed, mostly for practical reasons. We had proposed to use the reagent prepared from Cp_2TiCl_2 and zinc dust, which we had some good prior experience with. The challenge was the separation of titanium containing "gunk" from the product and remaining starting material. This is obviously a problem in any reaction using this reagent. However, the extent of the difficulty will depend on the structure of the substrate vs. the model compound used. With **1** the yields were variable. Some of the alternatives we investigated were the acid catalyzed opening to the diol **8** followed by *bis*-thiocarbonate formation and radical deoxygenation. In the end we isolated a sufficient amount of **8** from all of these reactions without having identified altogether satisfactory conditions that worked with **1** instead of just simple model compounds.



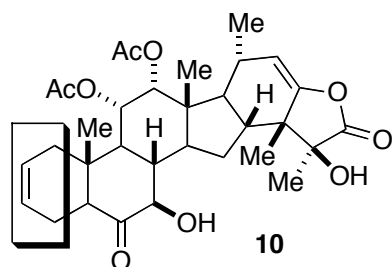
Multiple functionalization

In **Task 5** it was our intention to prepare compounds from which more than one functional group was deleted. We had planned that the bioassay information from the Cancer Center obtained on the analogs with a single structural modification would help us choose which derivatives to make. As mentioned above that did not work out as we had expected since the bioassay capability was lost.

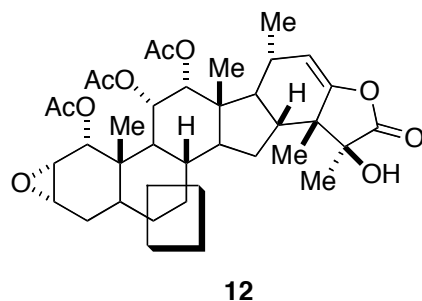
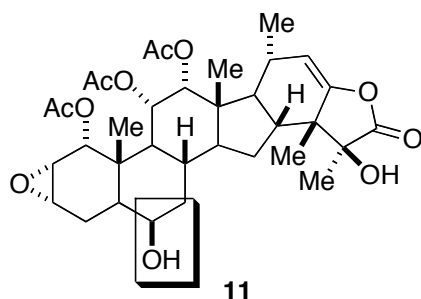
We therefore resorted to making derivatives based on the chemistry we had mastered by this point. Thus treatment of **1** with excess Sml_2 not only resulted in deoxygenation at C-7 but also in removal of the epoxide if the reaction was allowed to proceed for longer reaction time to yield **9**.



From compound **8** we also obtained compound **10** through catalytic hydrogenation with a poisoned Lindlar catalyst that is not capable of reducing either double bond in **8** when used in the presence of excess cyclohexene:



Starting from compound **6** we also prepared compounds **11** and **12** by reduction followed by radical deoxygenation to **12**.



To the best of our knowledge there are only two other species of *Tacca* growing in Hawaii, *Tacca palmata* and *Tacca leontopetaloides*. We had obtained small samples of the former in the course of our screening grant that led to the discovery of the taccalonolides, but the locally available plant population is not large enough that Arboretum personnel was willing to supply sufficient biomass for preparative extraction. *Tacca leontopetaloides* is also not readily available in Hawaii, although we obtained a few rhizomes from an ethnobotanical garden on the island of Hawaii. In this case as well insufficient material was available for the isolation preparatively useful amounts of material.

Although we explored importation of plant material from China and from Samoa, the restrictions for importation of live plants or plant parts into Hawaii made this not viable.

At the present time we have a small amount of taccalonolide E **4**, the 11-desacetoxyderivative of **1** in hand. Once we have bioassay results in hand for the derivatives of **1** we have prepared, I will use this material to prepare the 11-desacetoxy

analogs of the most potent compounds in the taccalonolide A series. This approach preserves precious compound and will allow us to establish whether there is a synergistic effect between modifications at C-11 and any other site.

Task 4 and **Task 6** were supposed to have been performed by our collaborators at CRCH. The Cancer Center is making efforts to rebuild the natural products program, which has recently moved to new laboratories in the medical school. In November of 2006 I have been able to hire a new technician to perform bioassays as part of the grants I am currently acting as a collaborator on. She has made excellent progress with the molecular assays we need for this work. She is a cell biologist by training and has expressed an interest in teaching herself the immunofluorescence assay for microtubule dynamics. She is located at the Cancer Research Center and has access to the tools that she will need for this work and I have secured some funding to buy out some of her time during the coming summer to do so.

Key Research Accomplishments

Procedures for the preparation of nine analogs of taccalonolide A have been worked out. These materials await biological testing for microtubule stabilizing activity.

Reportable Outcomes

None.

Conclusion

Loss of our bioassay capability and the slow pace at which I have been able to rebuild it have hampered progress of the projected work. Since the interest is not so much in the chemistry itself but in the biological effects of the modifications we have been able to perform, preparation of a publication describing our results depends on the bioassay. I think a solution is now at hand and I fully expect to have a manuscript to submit by the end of the summer break.

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Appendices

None